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Illumination-related pattern formations in lipid monolayers

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Abstract

We report on the phenomenon that in the two phase coexistence region (LC–LE) of a lipid monolayer film the fractal-like solid domains may grow simply by continuous illumination of a fluorescence microscope. The mechanism of this 2D domain growth is discussed. This phenomenon gives insight into the two-dimensional ramified crystallization in monolayers.

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Recently much attention has been paid to the two phase coexistence region of liquid condensed (LC) and liquid expanded (LE) phases in lipid monolayers [1–6]. In the LC phase the amphiphilic molecules are packed quite in order and all molecules are perpendicular to the water surface. In the LE phase, however, the molecules are more randomly distributed and there is neither universal molecular orientation nor positional order. The transition from the LE phase to the LC phase is actually an orientational phase transition and to some extent can be analogized to crystal growth from a solution. In previous experimental studies it has been found that the LC domains may nucleate and develop as faceted compact patterns [5,6], dendrites [4], fractals [3,7], and even more complicated morphologies. Sometimes the domain morphology may transit from one to another [5]. The investigations of the mechanism of LC domain growth, as well as the evolution process and selection principles between these patterns, are very active topics in both monolayer studies [1–6] and pattern formation research [8]. It is a challenge to interpret the development of the boundaries between the LC and LE phases with the existing theories of faceted crystal growth [9] and dendritic growth [8,10]. For example, it is known theoretically that a 2D crystal cannot be faceted. However, in previous monolayer studies, faceted LC domains have been observed [5,6]. Moreover, in a monolayer system, because of the dipole–dipole electrostatic interactions, the Mullius–Seckerka instability can be significantly modified at small wave vectors. It has been declared that this will have a dramatic effect on the dendritic growth in the monolayer system, leading to a maximum allowed size of the dendrite tip [10]. At present, the growth mechanism of LC domains in the LE–LC coexistence region seems

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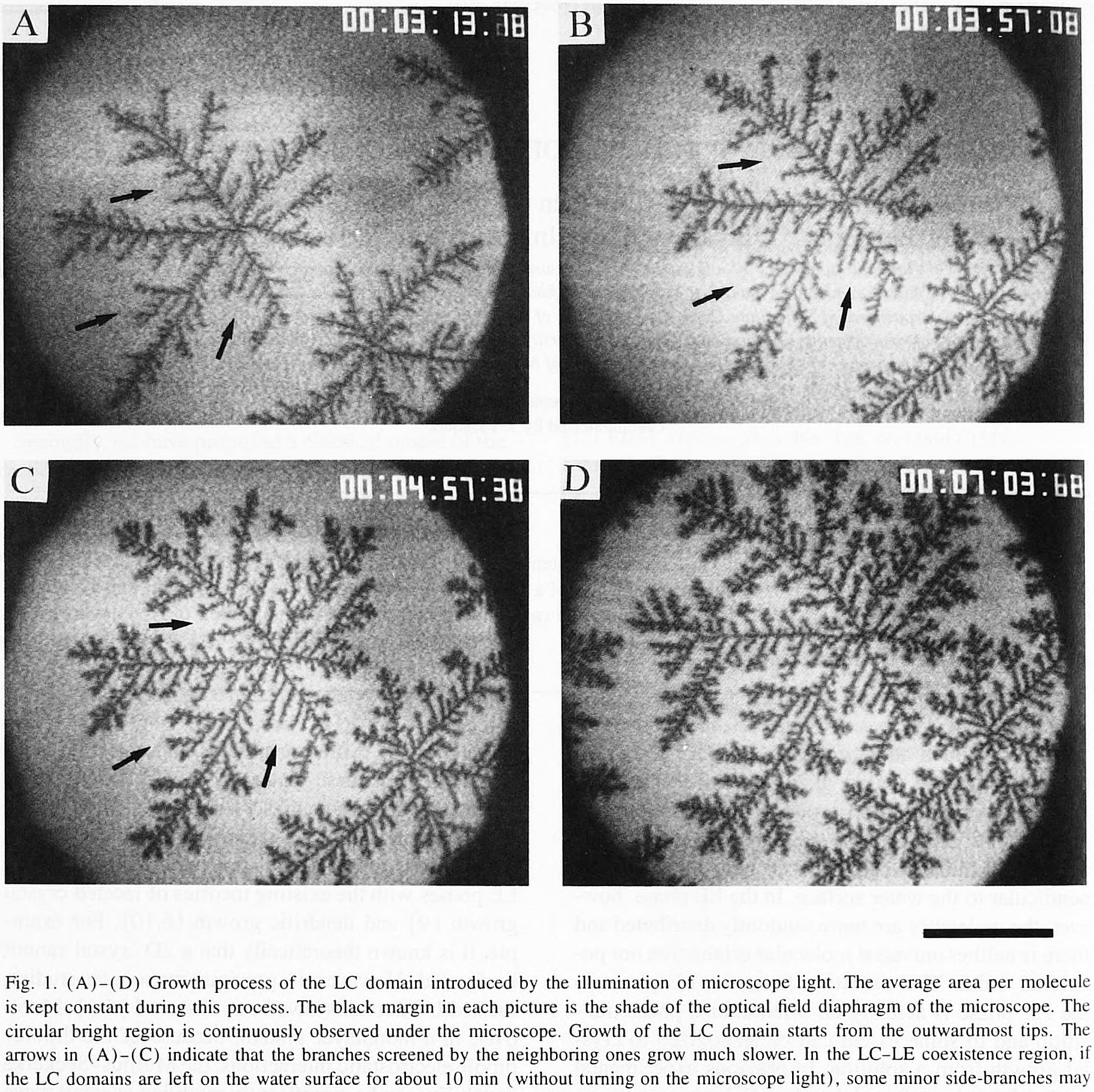


Fig. 1. (A)–(D) Growth process of the LC domain introduced by the illumination of microscope light. The average area per molecule is kept constant during this process. The black margin in each picture is the shade of the optical field diaphragm of the microscope. The circular bright region is continuously observed under the microscope. Growth of the LC domain starts from the outwardmost tips. The arrows in (A)–(C) indicate that the branches screened by the neighboring ones grow much slower. In the LC–LE coexistence region, if the LC domains are left on the water surface for about 10 min (without turning on the microscope light), some minor side-branches may shrink and only major branches are left on the air–water interface. The illumination-related growth also occurs to these shrunk domains, as indicated by (E)–(H). The figures in the time scale, from left to right, stand for hours, minutes, seconds and $\frac{1}{100}$ seconds, respectively. The bar represents 50 μm.

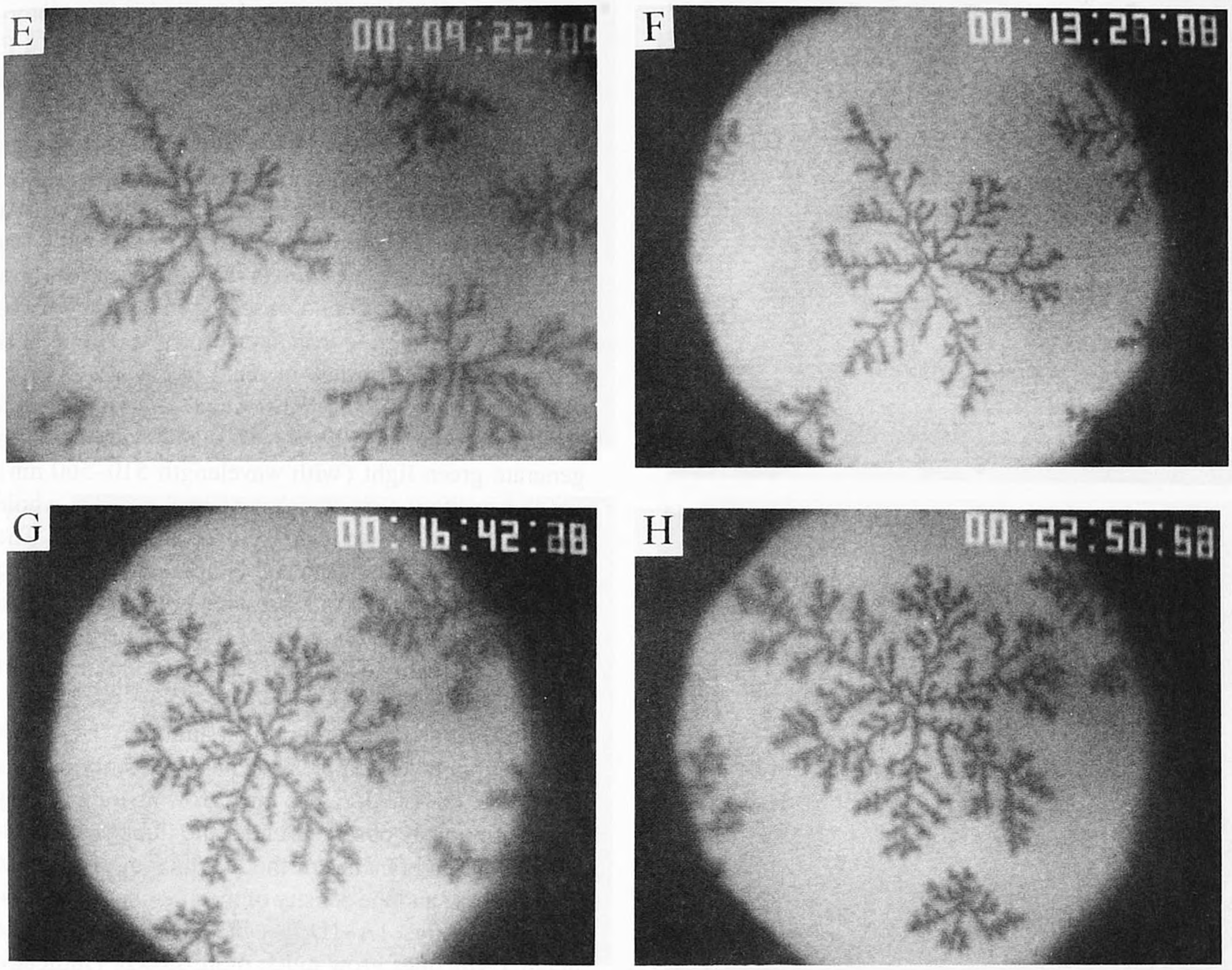
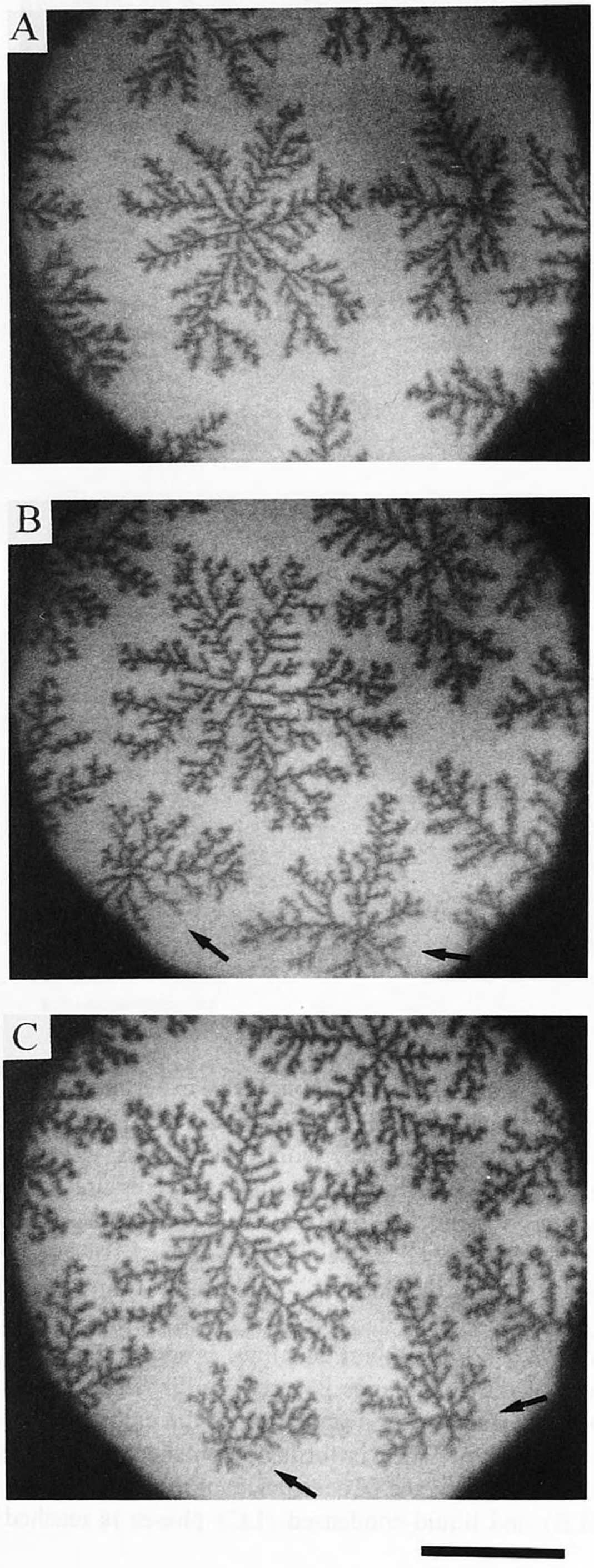


Fig. 1. (Continued)

still not very clear [7]. It is a question whether the growth of LC domains is controlled by "constitutional supercooling" [3] or "diffusion-limited aggregation" [7]. More experimental efforts will be helpful to give an answer. Here we report on the novel phenomenon that the domains in the liquid-condensed phase may grow under continuous illumination of microscope light, even though compression of the monolayer has been stopped for a long period and the average area per molecule at the air–water interface remains constant. The mechanism of such an illumination-related domain growth phenomenon is investigated. Inspired by this phenomenon, we propose a generic model for the domain growth in monolayers.

The trough for the monolayer experiment is a conventional one [3], which consists of a rectangular water pool coated with Teflon plates, a Teflon barrier and a Wilhelmy-type surface pressure measuring system. Millipore-filtered, ultrapure water (pH 5.5) is used as subphase. Synthesized surfactant N,N-dihexadecy-(1-imidazolyl)-propylamine (DIPA) [11] is dissolved in chloroform and 0.5 mol.% (DPPE-sulphorhodamine) is added. The mixture of the compounds is carefully dispersed on the water surface. After evaporation of the chloroform, a monolayer of DIPA is formed spontaneously on the air–water interface. Coexistence of liquid-expanded (LE) and liquid-condensed (LC) phases is reached



by compressing the monolayer film with the motor-controlled barrier. In the DIPA monolayers the LC–LE coexistence region is characterized by the formation of dark, fractal-like LC domains. When the LC domains become sufficiently large for observation, the barrier is stopped and fixed thereafter, and the water flow in the trough is allowed to be damped within a few minutes. The domains cease growing when an equilibrium surface pressure is reached. Meanwhile, a LC domain is chosen and observed under the microscope. By shifting the trough carefully, the domain is kept at the center of the viewfield of the microscope. The light source of the fluorescence microscope is a mercury arc lamp (100 W). A filter is employed to generate green light (with wavelength 510–560 nm) for the excitation of fluorescent probes. The whole trough is thermostated and kept at 20 ± 0.1°C. The trough, Wilhelmy balance and temperature measuring system are interfaced to a personal computer for data acquisition and system control. The growing process of LC domains is recorded by a microscope-matched video system.

To our surprise it is found that although the barrier for the monolayer compression has been stopped for a long time, the LC domains may grow as long as they are continuously observed under the fluorescence microscope. The branches of the LC domain extend into the LE phase and the density of the branches increases, as shown in Figs. 1A–1D. The branches trapped by the neighboring ones grow much more slowly (indicated by the arrows), which is known as screening effect, a typical feature of diffusion-limited growth. We want to emphasize that in order to avoid the possible local excess of surface pressure over the monolayer film, which may contribute to domain growth if it exists, we leave the LC domains on the water surface for some time. During this waiting period, the microscope light is switched off. Influenced by line tension, some minor sidebranches shrink and only backbones remain (Fig. 1E). Such a kind of shrinking is known as Oswald

Fig. 2. (A)–(C) show that the domains in the central part of the illuminated region are growing, while the branches in the shade of OFD are melting, as indicated by the arrows in (B) and (C). The domains indicated by the arrows were partly in the shade of the OFD. In order to see the morphology of the branches hidden in the shade of the OFD, the Langmuir trough was slightly shifted. The bar represents 50 μm.

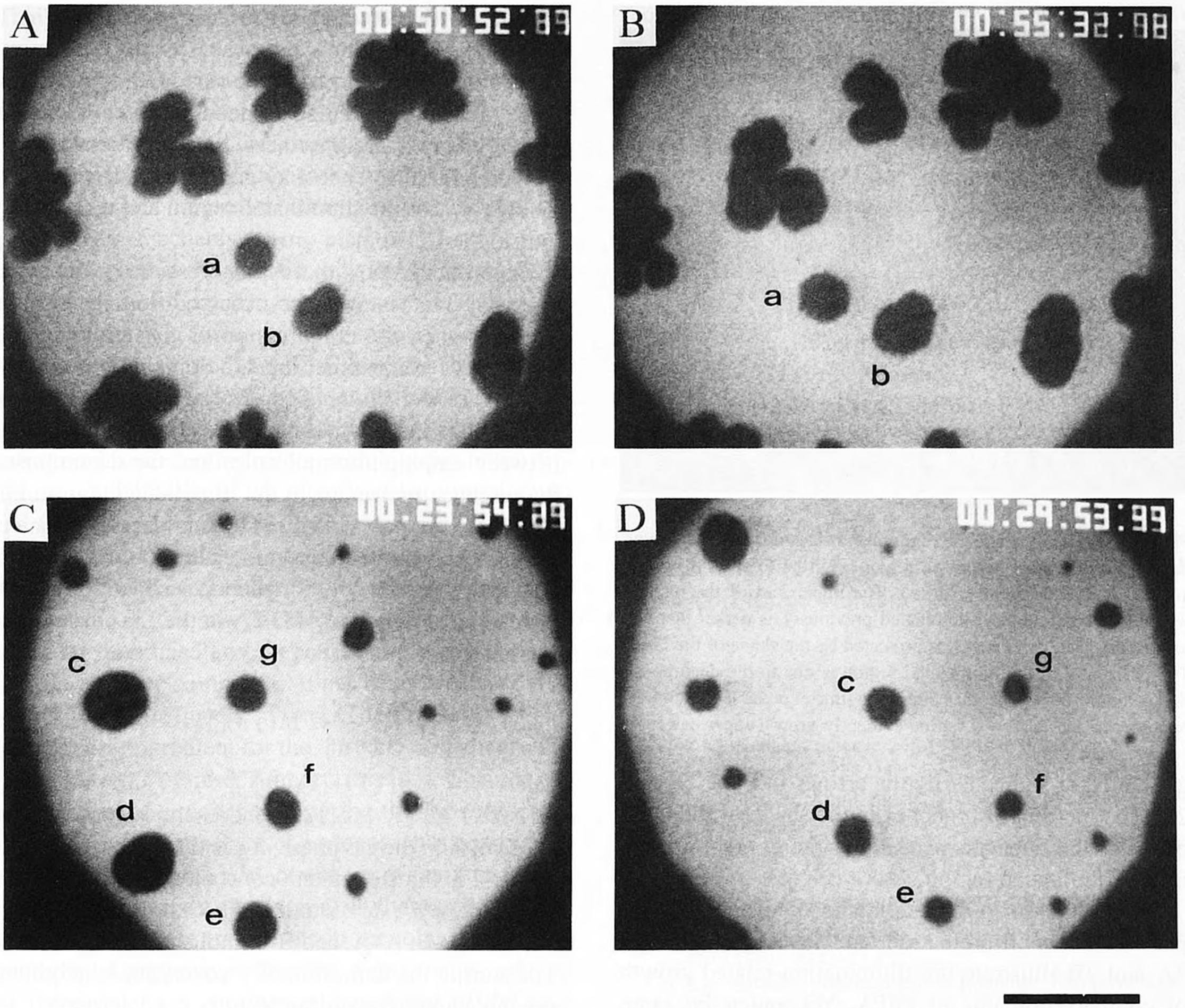


Fig. 3. Micrographs showing the different response of LC domains of different compounds when the domains are illuminated by the same microscope light source. (A), (B) Growth of compact LC domains of DIPA under continuous illumination. During this process the average area per molecule is kept at 0.60 nm^2/molecule. (C), (D), however, show the melting of circular LC domains of DMPE under illumination. The domains labeled by the letters correspond to each other in both (A), (B) and (C), (D). The experimental conditions, such as the average area per molecule, the temperature of the water subphase and the light intensity of the microscope, etc., are the same for the observations in (A), (B) and (C), (D). In the time scale, from left to right, the figures represent hours, minutes, seconds and $\frac{1}{100}$ seconds, respectively. The bar represents 50 μm.

ripening [12]. By observing the shrunk domains continuously, we find that the shrunk domains may also grow under illumination (Figs. 1E–1H). Therefore we conclude that the observed domain growth is not related to the plausible compression-induced imhomogeneous distribution of surface pressure. The screening effect shown in Fig. 1 implies that there exists a nutrient flux diffusing from the surrounding areas toward the growing cluster. The screening effect also suggests that the observed domain growth is not directly induced by photochemical reactions, otherwise growth would occur to all tips simultaneously. Actually it is expected that any absorption band of this compound must be below 250 nm [13].

We should point out that the growth occurs *only* to the branches illuminated by the microscope light (Fig. 2). Those branches in the shade of the optical field diaphragm (OFD) of the microscope are melting

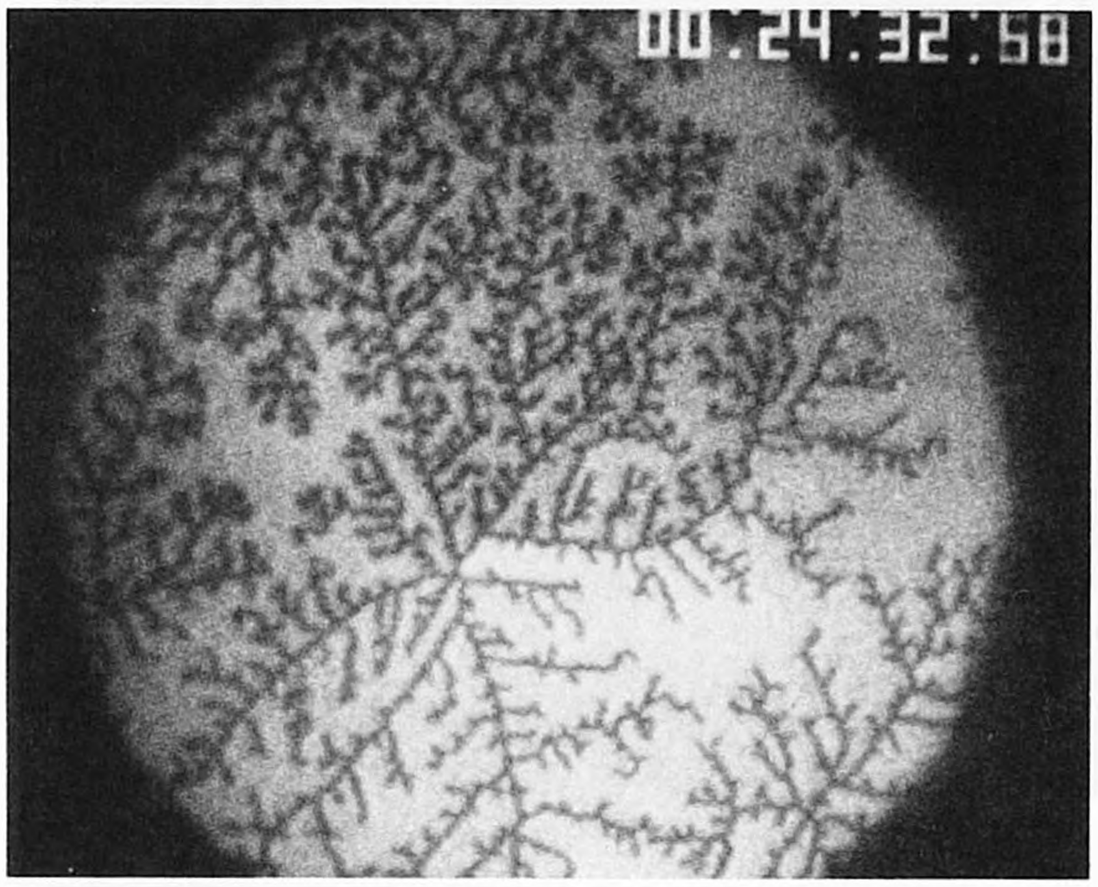


Fig. 4. The luminescence micrograph showing the situation around the boundary of the illuminated region. In order to see this boundary, the trough is slightly shifted. The upper part of the picture, which was continuously illuminated previously, is darker than the lower part. The lower part was protected by the shade of the OFD. The darkening of the illuminated region is due to the decomposition of fluorescent molecules after long time exposure. The domain branches in the darkened region evidently grow (upper part).

gradually, as indicated by the arrows in Figs. 2B and 2C. The melting of branches in the shade of the OFD provides the nutrients needed for the domain growth in the illuminated region.

The behaviors of LC domains of different compounds under illumination are investigated. Figs. 3A and 3B illustrate the illumination-related growth of compact domains of DIPA. Yet under the same experimental conditions, circular domains of L-α-dimyristoylphosphatidylethanolamine (DMPE) melt, as shown in Figs. 3C and 3D. A different response of LC domains of DIPA and DMPE under light illumination suggests that the illumination-related growth phenomenon depends on molecular structures and molecular interactions of the compounds.

Fig. 4 provides a hint to understand what has happened in the illuminated region. The darker region of Fig. 4 was illuminated by the microscope light, where the domain branches have become denser; while the brighter area was protected by the shade of the OFD, where branches melted gradually. It is known that the fluorescence probes may decompose when they are illuminated for a long period. When this happens the brightness in the view field will decrease. So it is reasonable to expect that in the dark region of Fig. 4 the decomposed fluorescent probes create a chemical environment different from the surrounding unilluminated areas and establish a chemical potential difference. In certain circumstances, this centripetal chemical potential difference may generate a mass flux diffusing towards the illuminated region and thus locally induce the LC domain growth.

Experiments show that the fluorescence probe itself does not contribute to the observed domain growth, because when the compression of the monolayer is finished for some time, the LC domains never grow if they are *not* illuminated. We suggest that the observed domain growth is controlled by the interactions between amphiphilic molecules and the decomposed fluorescence molecules in the illuminated region. For simplicity, we only consider DIPA molecules (A) and decomposed fluorescence molecules (B). Using the concept of regular solution theory, see also Ref. [14], the chemical potential of DIPA in the LE phase in the presence of impurity B, $[\mu_A]_{A,B}$ can be expressed as

$$[\mu_A]_{A,B} = [\mu_A]_A + kT \ln(\gamma X_A), \tag{1}$$

where $\gamma = \exp[(1 - X_A)^2 \sum_i^n \Phi_i/kT]$, $\Phi_i = \phi_i^{AB} - \frac{1}{2}(\phi_i^{AA} + \phi_i^{BB})$, $[\mu_A]_A$ is the chemical potential of pure DIPA in the LE phase, X_A is the molar fraction of DIPA, k is the Boltzmann constant and T is temperature. ϕ_i^{AB}, ϕ_i^{AA}, ϕ_i^{BB} stand for the interaction energies between A–B, A–A, and B–B molecules, respectively. The sum in the definition of γ covers the n neighboring molecules. According to (1)

$$\Delta\mu = [\mu_A]_{A,B} - [\mu_A]_A = kT \ln\left[X_A \exp\left((1 - X_A)^2 \sum_i \Phi_i/kT\right)\right]. \tag{2}$$

$\Delta\mu$ is the driving force for the amphiphilic molecules moving into or moving out of the illuminated region. The sign of $\Delta\mu$ decides the direction of molecule diffusion. If $\Delta\mu < 0$, the DIPA molecules will move into the illuminated region continuously, which increases the local molecular density and leads to the growth of LC domains. $\Delta\mu < 0$ corresponds to

$$\sum_i \Phi_i < \frac{kT}{(1 - X_A)^2} \ln(1/X_A). \tag{3}$$

This means that a stronger impurity-DIPA molecular interaction favors the growth of the LC domains. In the opposite situation, however, the compound molecules will diffuse out of the illuminated region. Consequently the LC domains in the illuminated region will melt in order to compensate the local decreasing of molecular concentration in the LE phase. This mechanism is supported by our recent observations that the domain growth rate increases as the concentration of the fluorescence probe and the illuminating light intensity are increased [15].

The growth mechanism of LC domains in monolayers was studied previously [3]. In the previous model the growth of LC domains was attributed to the constitutional supercooling effect, which is due to the difference in solubility of the impurities between the two existing phases and is well known in solidification. However, that model cannot explain the fact that the domain growth is also observed by Brewster angle microscopy [16], a new technique which does not require adding any fluorescent probes (impurity) into the amphiphilic compound. It has been suggested that impurities diffusing out of the LC domains may not be a necessary ingredient for the unstable domain growth [7]. Our report further supports this idea. There is actually a generic model to describe the growth of LC domains in monolayers. Let us denote the density field of amphiphilic molecules on the air–water interface as $\rho(r,t)$. We suggest that $\rho(r,t)$ is the parameter that controls the phase transitions in monolayers. In conventional Langmuir monolayer experiments, $\rho(r,t)$ is increased by a continuous decreasing of the area of the monolayer film. In our experiment, however, $\rho(r,t)$ is varied by the local mass transport driven by the inhomogeneous distribution of the chemical potential. Whatever the method people used to increase $\rho(r,t)$, as soon as a critical density ρ_c is reached, nucleation of LC domains occurs and the LC phase coexists with the LE phase. Meanwhile, a further increase of $\rho(r,t)$ may create a supersaturation, which acts as a driving force of LC domain growth. Analogous to crystal growth from a solution, the development of LC domains can be described by a diffusion equation, $\mathrm{d}\rho(r,t)/\mathrm{d}t = \nabla^2\rho(r,t)$. The dimensionless driving force for the domain growth, $\Delta\mu/kT$, can then be expressed as

$$\frac{\Delta\mu}{kT} \propto \ln\left(\frac{\rho(r,t)}{\rho_c}\right). \tag{4}$$

During the growth of LC domains, as soon as the molecular density $\rho(r,t)$ becomes larger than ρ_c, the LC domains will grow. In conventional Langmuir monolayer experiments, this condition is guaranteed by continuously decreasing the area of the monolayer film. In the case of illumination-related growth, it is realized by the local mass transfer. Up to now, it seems that all the observed LC domain growth phenomena in monolayers can be interpreted by this model.

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